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FINAL REPORT

Title Page

Project No. (6X61-13-001 - Investigations to Determine the Effects of Insecticides (Including Chemosterilants) on Insects Infected with Pathogens)

Task No. 1 (The Effects of a Chemosterilant on Malaria in Mosquitoe

Name and Address of Reporting Installation:

Orlando Laboratory, Entomology Research Division
Agricultural Research Service, U. S. Department of Agriculture
500 Primrose Drive,
Orlando, Florida

Period Covered by the Report: 20 February, 1962 - 30 November, 1962

Professional Authors of the Report:
Principal Investigator: Major Robert M. Altman, MSC

Reports Control Symbol: (RCS - MEDDH - 288)

Security Classification: Unclassified

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ABSTRACT

Project No. 6X61-13-001 Title: Ecology and Control of Disease Vectors and Reservoirs

Task No. 1 Title: The Effects of a Chemosterilant on Malaria in Mosquitoes

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Studies were conducted to determine the effects of the chemosterilant tepa on Plasmodium gallinaceum in Aedes aegypti. It was demonstrated that holding the mosquitoes on a tepa residue of 10 mg./sq. ft. either immediately before or after they fed on infected chicks caused a reduction in the percentage of mosquitoes that became infected and a reduction in the mean oöcyst count. Similar exposures also caused reductions in malaria transmission rates. Other experiments demonstrated that tepa reduced the malaria transmission rates when mosquitoes infected for more than 14 days were held on a tepa residue of 10 mg./sq. ft. Malaria did not develop in 35 of 36 mosquitoes that fed on infected chicks one hour after they were inoculated intraperitoneally with tepa at the rate of 100 mg./kg. Malaria developed in chicks following intramuscular inoculation with blood samples taken from infected chicks one hour after they were inoculated intraperitoneally with tepa.

Project No. 6X61-13-001 Title: Investigations to determine the effects of insecticides (including chemosterilants) on insects infected with pathogens.

Task No. 1 Title: The effects of a chemosterilant on malaria in mosquitoes.

Description:

The specific aim of this research was to determine if a chemosterilant could affect the multiplication or development (or both) of Plasmodium gallinaceum within Aedes aegypti.

Progress:

It has been demonstrated that insects can be sterilized by chemicals which collectively have been labeled chemosterilants (LaBrecque, 1961). The alklating agents tepa (tris(1-aziridinyl)phosphine oxide), metepa (tris(2-methyl-1-aziridinyl)phosphine oxide), and apholate (2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine) have been investigated more thoroughly than the other materials and small dosages will sterilize house flies (Musca domestica L.), Aedes aegypti (L.), and Anopheles quadrimaculatus Say (Weidhaas et al., 1961; LaBrecque et

al., 1962; Weidhaas, 1962; Eddy et al., unpublished data). A. aegypti can be sterilized by tepa when they are exposed in the larval stage, when adults are held on a treated surface, when adults are fed sweetened water baits containing the chemical, and when they are allowed to engorge on animals that have been inoculated intraperitoneally. The present study was conducted to determine the effect of tepa on Plasmodium gallinaceum Brumpt in A. aegypti.

The host mosquitoes were the Orlando strain of A. aegypti. They were infected when they were from 6 to 9 days old and thereafter were maintained on a 5% sugar solution and held at approximately 27° C. and 80% relative humidity. The mosquitoes were anesthetized with ether and/or immobilized by holding them at 2° C. so that they could be manipulated. In each experiment mosquitoes for a control group were taken from the same brood as those exposed to tepa and both groups were held in the same constant temperature box.

Fresh lots of tepa were obtained monthly. The concentrated chemical was stored at 2° C. and was diluted on the day it was used. The tepa was dissolved in absolute methanol and applied evenly to the inside of a 1-pint glass jar. A glass petri dish was treated at the same time to use as a cover on the jar while the mosquitoes were being exposed. The jar and petri dish were air-dried at approximately 27° C. for 4 to 22 hours before they were used. In all the experiments the glass surface was treated with approximately 10 mg. of tepa per square foot. The tepa was bioassayed in each experiment by collecting eggs from the mosquitoes that had been exposed and testing them for viability.

A heavy breed of chickens was used in these studies. The donor chicks were inoculated intramuscularly with P. gallinaceum (strain 8A) and used to infect the mosquitoes when the parasitemia approximated 20%. Blood smears were made from chicks used in transmission experiments 8, 9, and 10 days after they were bitten by mosquitoes.

Oocyst counts were made of a representative number of mosquitoes from each experiment 8 days after they were infected. Transmission attempts were made 14 or more days after the mosquitoes were infected.

Development of malaria in mosquitoes infected and subsequently held on a tepa residue.

Seven experiments were conducted to determine the effects of tepa on P. gallinaceum in A. aegypti when the mosquitoes were given an infectious blood meal and subsequently held for varying periods of time on glass treated with approximately 10 mg./sq. ft. In six of the experiments the engorged mosquitoes were put on the tepa in less than 20 minutes; in experiment number 7 the engorged mosquitoes were put on the tepa residue 45 minutes after feeding. The feeding time did not exceed 1 hour in any experiment. The results of these experiments are shown in table 1.

In each experiment mosquitoes from the same brood were infected on the donor chick approximately 30 minutes after the tepa-treated mosquitoes and used as controls.

To determine if the parasite was developing in the mosquitoes, samples were removed from the cages 8 days after their infective meal and gut dissections for oöcyst counts were made. In three experiments there were no oöcysts in mosquitoes that had been exposed to tepa. In the other four experiments there was a lower rate of infection and a smaller mean oöcyst count in the tepa-treated mosquitoes than in the controls. The majority of the oöcysts in the tepa-treated mosquitoes were smaller than those in the control mosquitoes. The results of the dissections are shown in table 1.

Transmission attempts were made with individual mosquitoes from the control groups. In some instances the transmission attempts with the treated mosquitoes were by multiple feedings. In four of the five experiments where transmissions were attempted, malaria was transmitted by at least 50% of the control mosquitoes, whereas there was no transmission by those from the tepa-treated group. In the other experiment malaria was transmitted by mosquitoes from the tepa-treated pool, but the rate was much lower than that obtained with mosquitoes from the control group. The results of the transmission attempts are shown in table 1.

In all these experiments nonviable eggs were collected from the mosquitoes exposed to tepa, demonstrating that the chemical was biologically active. Viable eggs were collected from all groups of control mosquitoes.

In two of the experiments there was high mortality in the tepa-treated groups, both in the exposure jars and in the holding cages. In the other experiments tepa did not produce any obvious deleterious effects.

Development of malaria in mosquitoes held on a tepa residue and subsequently infected.

Five experiments were conducted to determine the effects of tepa on P. gallinaceum in A. aegypti where the mosquitoes were held for varying periods of time on glass treated at the rate of 10 mg./ sq. ft and given an infectious blood meal immediately afterwards. The results of these experiments are shown in table 2.

In each experiment mosquitoes from the same brood were infected on the donor chick approximately 2 hours before the tepa-treated mosquitoes and used as controls.

Gut dissections for oöcyst counts were made 8 days after the infective meal. In all the experiments there was a lower rate of infection and a smaller mean oöcyst count in the tepa-treated mosquitoes than in the control mosquitoes. The majority of the oöcysts in the tepa-treated mosquitoes were smaller than those in the controls. The results of the dissections are shown in table 2.

Transmission attempts were made with individual mosquitoes from the control groups (in some experiments the control mosquitoes were those that had been reported in the experiments shown in table 1).

The transmission attempts with the treated mosquitoes were by single and multiple feedings. In one of the experiments 21 of 38 mosquitoes from the control group transmitted malaria whereas there was no transmission in the attempts with tepa-treated mosquitoes. In the other two experiments malaria was transmitted by the tepa-treated mosquitoes, but the rate of transmission was lower than that

obtained with mosquitoes from the controls. The results of these transmission attempts are shown in table 2.

In all these experiments the mosquitoes exposed to tepa did not produce eggs. Viable eggs were collected from all groups of control mosquitoes.

The mosquito mortality was high in the tepa-treated jar in one of the experiments, but the survival in the holding cage was normal. In the other experiments the tepa treatment caused a reduction in the number that would bite and some mortality in the exposure jars, but the survival in the holding cage was normal.

The effect of tepa on sporozoites in the salivary glands of mosquitoes.

Table 3 gives a summary of five experiments conducted to ascertain the effect of tepa on sporozoites in the salivary glands of infected mosquitoes when they were held for varying periods of time on glass treated with approximately 10 mg./sq.ft. The mosquitoes used in these experiments were selected from the controls of the experiments that have been reported previously and had been infected 14 or more days.

Transmission attempts were made with individual mosquitoes from both the tepa-treated and control groups. The tepa-treated mosquitoes were offered a recipient chick from 20 to 120 minutes after they were removed from the treatment jars and the mosquitoes that fed did so immediately. Malaria was transmitted by at least one mosquito from each group that had been exposed to tepa. The rate of transmission by mosquitoes from the tepa-treated groups was lower than their respective controls.

In experiments 13, 14, and 15 there was high mortality in the treatment jars and a poor biting rate with the mosquitoes exposed to tepa. In the other two experiments there was low mortality and a fair biting rate (17/20 fed in experiment number 16 and 12/20 fed in experiment number 17).

The tepa residue was bioassayed after the infected mosquitoes had been removed. This was done by holding engorged mated 6-day-old A. aegypti in the treatment jars for approximately 3 hours after the infected mosquitoes had been exposed. No viable eggs were collected from these mosquitoes.

The effects of tepa on malaria in mosquitoes following the simultaneous ingestion of tepa and blood from infected birds.

Two experiments were conducted to determine if P. gallinaceum would develop in mosquitoes that engorged on infected birds previously inoculated with tepa.

In these experiments pools of mosquitoes for controls were fed on donor chicks with a parasitemia of approximately 30%. Immediately afterward the chicks were inoculated intraperitoneally with an aqueous solution of tepa at the approximate rate of 100 mg./kg. One hour after inoculation, A. aegypti from the same broods as the controls were allowed to fed on the donor chicks for 20 to 30 minutes.

Oöcyst counts were made of mosquitoes from each experiment 8 days after the infective meal. In one of the experiments there was no oöcysts in the mosquitoes that had fed on an infected chick after it had been inoculated with tepa. In the other experiment 3

small oöcysts were found in one of 16 mosquitoes that had fed on an infected chick after it had been inoculated with tepa. The development of oöcysts in the mosquitoes from the control pools was consistent with that observed in the other controls in this study. The results of the dissections are shown in table 4.

In one experiment the donor chick was bled with a needle and syringe wet with heparin after the second lot of mosquitoes had completed feeding. This was approximately 1-1/2 hours after the chick had been inoculated with tepa. Five 2-week-old chicks were each inoculated intramuscularly with 0.2 ml. of the blood. Malaria developed in all five of the chicks.

Eggs were collected from the mosquitoes to bioassay the tepa. Less than 0.5% of the eggs from the tepa-treated mosquitoes hatched while there was a normal hatch of the eggs from the control mosquitoes.

At a later date two other donor chicks with a parasitemia of approximately 40% were inoculated intraperitoneally with an aqueous solution of tepa at the rate of 100 mg./kg. One hour after inoculation the chicks were bled and four 2-week-old chicks were inoculated intramuscularly with 0.2 ml. of blood from each donor bird. Malaria developed in all eight of the chicks.

Summary and Conclusions:

Studies were conducted to determine the effects of the chemosterilant tepa on Plasmodium gallinaceum in Aedes aegypti. It was demonstrated that holding the mosquitoes on a tepa residue of 10 mg./sq. ft. either immediately before or after they fed on infected chicks caused a reduction in the percentage of mosquitoes that became infected and a reduction in the mean oocyst count. Similar exposures also caused reductions in malaria transmission rates. Other experiments demonstrated that tepa reduced the malaria transmission rates when mosquitoes infected for more than 14 days were held on a tepa residue of 10 mg./sq. ft. Malaria did not develop in 35 of 36 mosquitoes that fed on infected chicks one hour after they were inoculated intraperitoneally with tepa at the rate of 100 mg./kg. Malaria developed in chicks following intramuscular inoculation with blood samples taken from infected chicks one hour after they were inoculated intraperitoneally with tepa.

These experiments were designed after learning that mosquitoes could be sterilized by resting on surfaces treated with chemosterilants. It was reasoned that sufficient chemical might be picked up to prevent the development of the parasite within the mosquito. These experiments proved that assumption to be correct.

Additional experiments are needed to determine how the chemosterilant affects the Plasmodium. The mode of action probably varies with the different experiments. Where the insects are held on a treated surface immediately before or after the infective blood meal some phase of the sexual development is probably interrupted.

The action of the tepa on the sporozoites in the salivary glands is possibly different from the above. The effects of the tepa when infected birds are inoculated intraperitoneally presents still other questions for it may have affected the gametocyte in the bird or may interfere with the sexual development of the malaria in the mosquito.

The present study is not sufficiently definitive to form any conclusions as to the practical aspects of malaria control with chemosterilants. The results of these experiments do, however, lend support to the theory presented by Lindquist (1961) that more rapid control of insect-borne diseases might be obtained if a sterilizing agent could be developed that would also act directly on the parasite. The techniques used in this study provide new methods of investigations for tepa, metepa, and apholate are radiomimetic and can be used to replace conventional methods of irradiation.

List of Publications:

(1) The Effects of Tepa on Plasmodium gallinaceum in Aedes aegypti. Manuscript submitted to "The American Journal of Hygiene" for publication on 19 November 1962.

(2) A paper with the same title presented 4 December 1962 at the annual meeting of the Entomological Society of America in Phoenix, Arizona.

Table 1.--The effects of tepa on Plasmodium gallinaceum in Aedes aegypti when the mosquitoes were infected, then held on glass treated with tepa at 10 mg./sq. ft.

Experiment Number	Hours Exposed	Mosquito Dissections: Control: Number Dissected	Control: Number Infected	Control: Percent Infected	Control: Mean Oöcyst Count	Tepa Treatment: Number Dissected	Tepa Treatment: Number Infected	Tepa Treatment: Percent Infected	Tepa Treatment: Mean Oöcyst Count	Transmissions: Control	Transmissions: Tepa Treatment
1	9	11	5	45	4	6	0	0	0	3/4**	0/5
2	4 2/3	15	7	47	55	10	0	0	0	No transmissions attempted	
3	5	9	7	78	42	10	0	0	0	No transmissions attempted	
4	4	20	16	80	17	21	4	19	2*	21/38	0/24
5	4 1/4	12	12	100	32	10	8	80	9*	12/24	4/26
6	4	20	20	100	29	21	16	76	19*	14/19	0/10
7	4 3/4	15	14	93	41	16	6	38	9*	42/52	0/20

*Most of the oöcysts were very small.

**Numerator = number positive, denominator = number feedings.

Table 2.--The effects of tepa on Plasmodium gallinaceum in Aedes aegypti when the mosquitoes were held on glass treated at 10 mg./sq. ft., then infected.

Experiment Number	Hours Exposed	Mosquito Dissections: Control: Number Dissected	Control: Number Infected	Control: Percent Infected	Control: Mean Oöcyst Count	Tepa Treatment: Number Dissected	Tepa Treatment: Number Infected	Tepa Treatment: Percent Infected	Tepa Treatment: Mean Oöcyst Count	Transmissions: Control	Transmissions: Tepa Treatment
8	4	9	7	78	42	19	12	63	6*	No transmissions attempted	
9	2 3/4	20	16	80	17	11	3	27	2*	21/38**	0/10
10	3	12	12	100	32	10	4	40	1*	12/24	3/18
11	4	20	20	100	29	15	8	53	9*	14/19	2/10
12	4	16	15	94	30	10	5	50	3*	No transmissions attempted	

*Most of the oöcysts were very small.

**Numerator = number transmissions, denominator = number feedings.

Table 3.--Results of transmission experiments to determine the effects of tepa on sporozoites of *Plasmodium gallinaceum* in the salivary glands of *Aedes aegypti*.

Experiment Number	Hours Exposed	Time after exposure when transmission was attempted	Controls	Tepa Treatment
13	3	20 min.	8/13*	1/4
14	1 1/2	120 min.	Same as Expt. 13	1/7
15	2	30 min.	10/16	1/2
16	1 1/2	45 min.	14/19	7/17
17	2	45 min.	14/18	5/12

*Numerator = number transmissions, denominator = number feedings.

Table 4.--Results of mosquito dissections eight days after Aedes aegypti had fed on chicks infected with Plasmodium gallinaceum that had been inoculated intraperitoneally with tepa.

Experiment Number	Control				Tepa Treatment			
	Number Dissected	Number Infected	Percent Infected	Mean Oöcyst Count	Number Dissected	Number Infected	Percent Infected	Mean Oöcyst Count
18	19	18	95	40	20	0	0	0
19	20	20	100	28	16	1*	6	<1

*The three oöcysts were very small.

References Cited

1. LaBrecque, G. C. 1961. Studies with three alkylating agents as house fly sterilants. Journal of Economic Entomology 54(4): 684-689.

2. LaBrecque, G. C., D. W. Meifert, and Carroll N. Smith. 1962. Mating Competitiveness of Chemosterilized and Normal Male House Flies. Science 136: 388-389.

3. Lindquist, A. W. 1961. Chemicals to sterilize insects. Journal of the Washington Academy of Sciences Nov. 1961: 109-114.

4. Weidhaas, D. E., H. R. Ford, James B. Gahan, and Carroll N. Smith. 1961. Preliminary observations on chemosterilization of mosquitoes. Proceedings 48th Annual Meeting New Jersey Mosquito Extermination Association.

5. Weidhaas, D. E. 1962. Chemical sterilization of mosquitoes. Nature 195: 786-787.

Project No. 6X61-13-001, Ecology and Control Disease Vectors and Reservoirs

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